B. SIMPULAN DAN SARAN

A. Simpulan

1. Ekstrak tanaman obat *Nan Fei Shu* tidak memiliki aktivitas antifungi terhadap *Candida albicans*.
2. Penamaan ilmiah tanaman obat *Nan Fei Shu* yakni *Vernonia amygdalina* atau *Gymnanthemum amygdalinum.*

B. Saran

1. Ekstrak *crude* perlu dilakukan isolasi senyawa fitol dan neophytadiene dengan kromatografi cair seperti HPLC untuk menguji potensi senyawa tersebut dalam menghambat *Candida albicans*.
2. Kultur yang digunakan untuk pengujian aktivitas antifungi direkomendasikan berasal dari *genuine American Type Culture Collection* dan selanjutnya penggunaan kultur maksimal berasal dari subkultur *genuine American Type Culture Collection* (ATCC) yang kelima.
3. Ekstraksi perlu dilakukan dari bahan simplisia segar menggunakan metode perkolasi dengan pelarut non polar seperti petroleum eter dan heksana terhadap *Aspergillus niger*, *Aspergillus fumigatus*, dan *Aspergillus flavus.*

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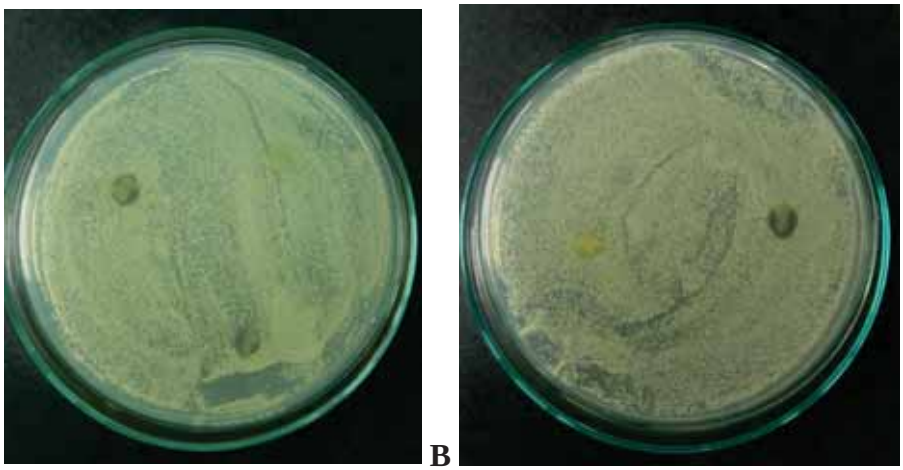
LAMPIRAN 1

Gambar 9. Tanaman obat *Nan Fei Shu*

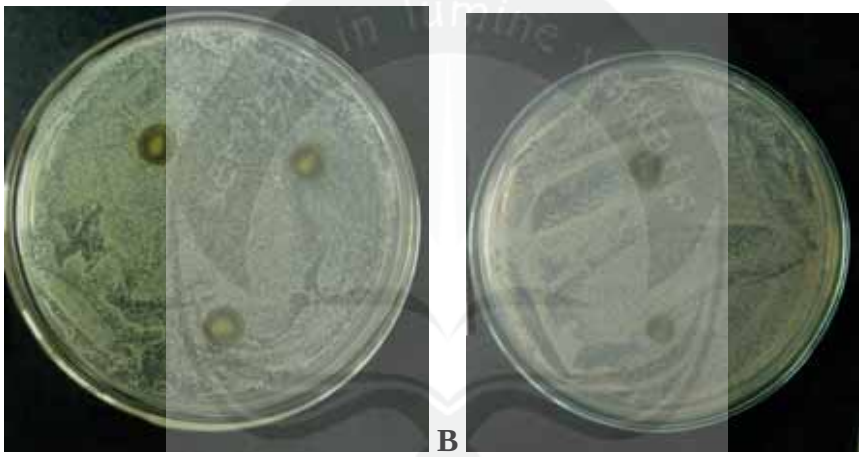
Gambar 10. Penampang daun tanaman obat *Nan Fei Shu*

Gambar 11. Beragam ukuran daun tanaman obat *Nan Fei Shu* dari termuda (kanan) hingga sebelum layu (kiri) diatas kertas berukuran A4.



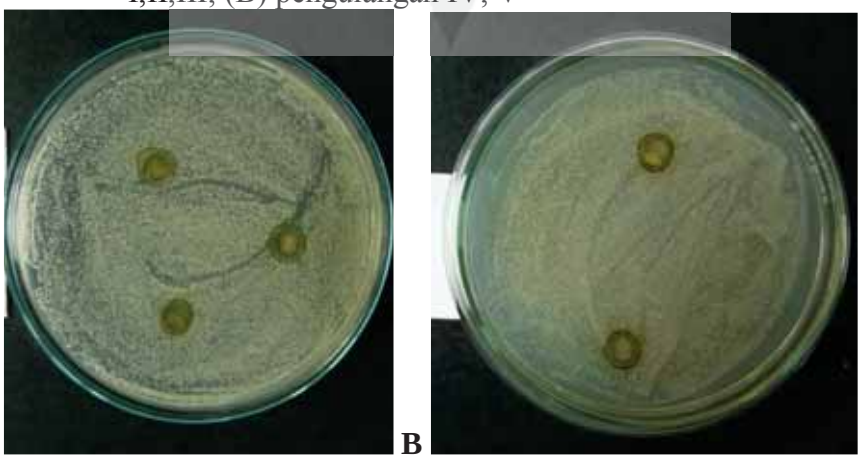
A B

Gambar 11. Uji zona hambat pada konsentrasi 200 mg/ml. (A) pengulangan I,II,III; (B) pengulangan IV, V



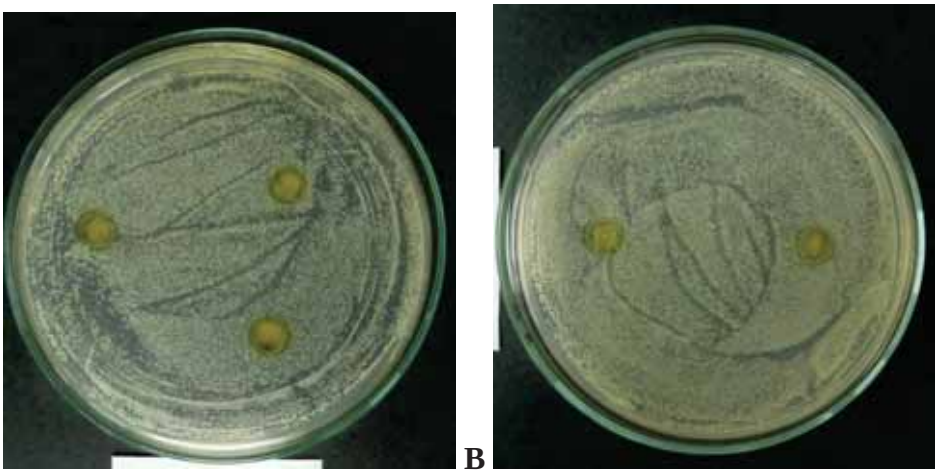
A B

Gambar 12. Uji zona hambat pada konsentrasi 100 mg/ml. (A) pengulangan I,II,III; (B) pengulangan IV, V



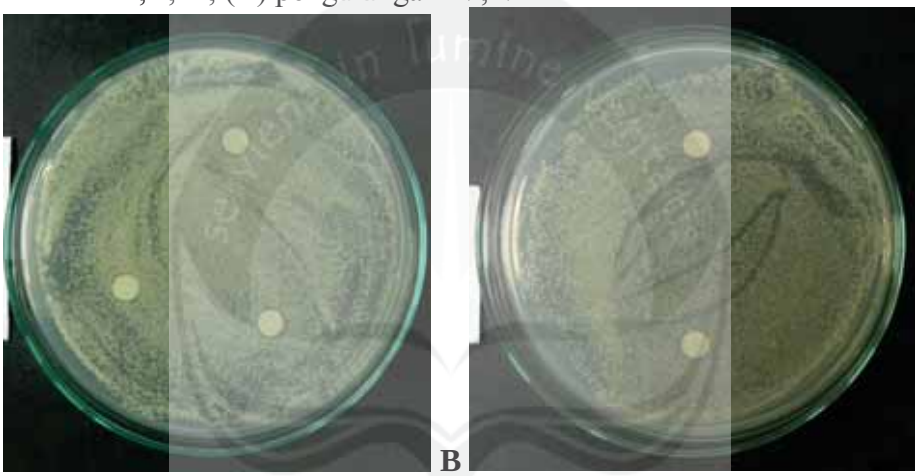
A B

Gambar 13. Uji zona hambat pada konsentrasi 50 mg/ml. (A) pengulangan I,II,III; (B) pengulangan IV, V



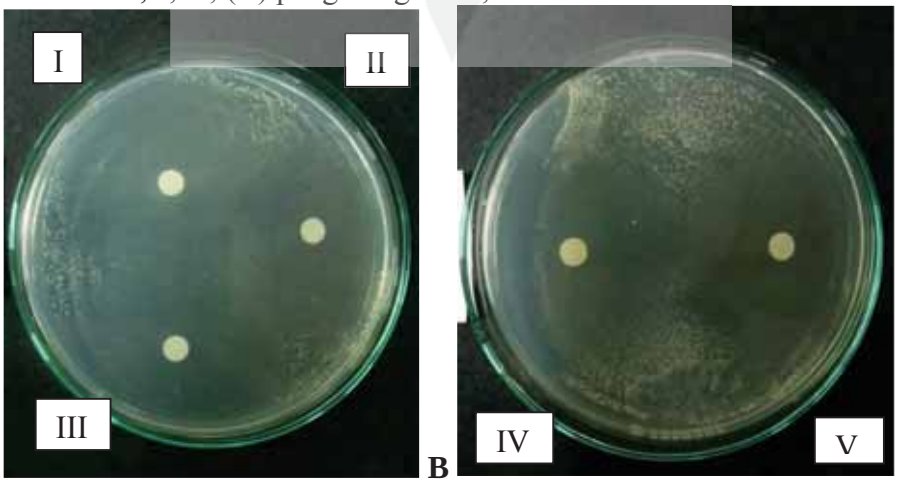
A B

Gambar 14. Uji zona hambat pada konsentrasi 25 mg/ml. (A) pengulangan I,II,III; (B) pengulangan IV, V



A B

Gambar 15. Uji zona hambat pada kontrol negatif, DMSO (A) pengulangan I,II,III; (B) pengulangan IV, V



A B

Gambar 16. Uji zona hambat pada kontrol positif, ketoconazole (A) pengulangan I,II,III; (B) pengulangan IV, V

LAMPIRAN 2

Tabel 3. Hasil identifikasi KG-SM ekstrak tanaman obat *Nan Fei Shu*

Puncak ke-	Waktu Retensi (menit)	Komponen	Persentase (%)
23	16,694	phytol	15,397
36	24,244	heneicosane	11,009
18	13,997	neophytadiene	9,885
22	15,33	hexadecanoic acid	8,959
24	17,026	9,12-octadecadienoic acid	8,571
32	22,786	nonacosane	4,591
14	13,328	cis-2-ethyl-2-hexen-1-ol	3,508
42	26,841	chondrillasterol	3,015
20	14,438	neophytadiene	2,962
15	13,552	pentacosane	2,882
16	13,63	(7e)-1,8-dimethyl-7-dodecenyl acetate	2,328
17	13,782	pluchidiol	1,951
41	26,427	2-methyl-7-phenylindole	1,872
40	26,185	2-ethylacridine	1,728
19	14,249	neophytadiene	1,486
37	24,765	alpha -tocopherol	1,26
3	9,859	3-buten-2-one,4-(1,3,3-trimethyl-7-oxabicyclo[4.1.0]hep-2-yl)-,[1.alpha.,2.beta.(z),6.alpha.]	1,198
29	22,36	1,5,9-undecatriene,2,6,10-trimethyl-	1,137
39	25,623	methyl (4-tert-butylphenoxy) acetate	1,098
46	28,058	1,2-bis(trimethylsilyl)benzene	1,094
48	29,772	1,2-bis(trimethylsilyl)benzene	1,092
13	12,842	3,6-octadien-1-ol,3,7-dimethyl-	0,876
35	24,088	beta -tocopherol	0,86
49	33,566	2-ethylacridine	0,799
10	12,19	chloromethyl 7-chlorononanoate	0,745
47	29,01	1,2-bis(trimethylsilyl)benzene	0,74
9	11,655	1-(2-methylcyclopent-1-enyl)-1-hydroxy-2-propene	0,69
11	12,365	(trans)-2-azidocyclopentan-1-ol	0,689
21	14,85	triacontane	0,627
34	23,468	eicosane	0,608
50	33,884	n-cyano-n',n',n'',n''-tetramethyl-1,3,5-triazinetriamine	0,577
25	17,607	5-nitrouracil	0,452
12	12,615	nonadecane	0,427
2	9,204	1,4-diethylhexyl methoxyacetate	0,379
45	27,658	benzo[h]quinoline, 2,4-dimethyl-	0,379

6	10,861	2(4h)-benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	0,358
8	11,531	2-piperidinone	0,338
43	27,082	2-ethylacridine	0,328
27	21,386	eicosane	0,319
7	10,961	propane	0,318
26	20,353	1-naphthalenamine-5,8-13c2,5,6,7-tetrahydro-	0,316
31	22,614	2-methyl-7-phenylindole	0,306
33	23,304	2-ethylacridine	0,304
1	9,033	2,3,5,-trimethylhexane	0,286
5	10,127	nonadecane	0,263
44	27,243	cyclotrisiloxane, hexamethyl-	0,248
4	10,071	1,5-dimethylcyclohexene-5-carbocaldehyde	0,243
28	22,098	2-nonadecanone	0,22
30	22,471	benzoic acid,2,4-dimethoxy-6-propyl-,4-carboxy-3-hydroxy-5-propylphenyl ester	0,142
38	25,109	4'methyl-2phenylindole	0,14

LAMPIRAN 3

Tabel 4. Hasil analisis variasi (ANOVA) luas zona hambat aktivitas antifungal variasi konsentrasi ekstrak tanaman obat *Nan Fei Shu*, ketoconazole 1% ($^{b}/_{v}$), dan DMSO terhadap *Candida albicans*.

	Jumlah (cm^2)	df	Rerata (cm^2)	F	Sig.
Antar Kelompok	546,165	5	109,233	274,600	,000
Dalam Kelompok	9,547	24	0,398		
Total	555,712	29			

Tabel 5. Hasil Duncan letak beda nyata luas zona hambat aktivitas antifungal variasi konsentrasi ekstrak tanaman obat *Nan Fei Shu*, ketoconazole 1% ($^{b}/_{v}$), dan DMSO terhadap *Candida albicans*.

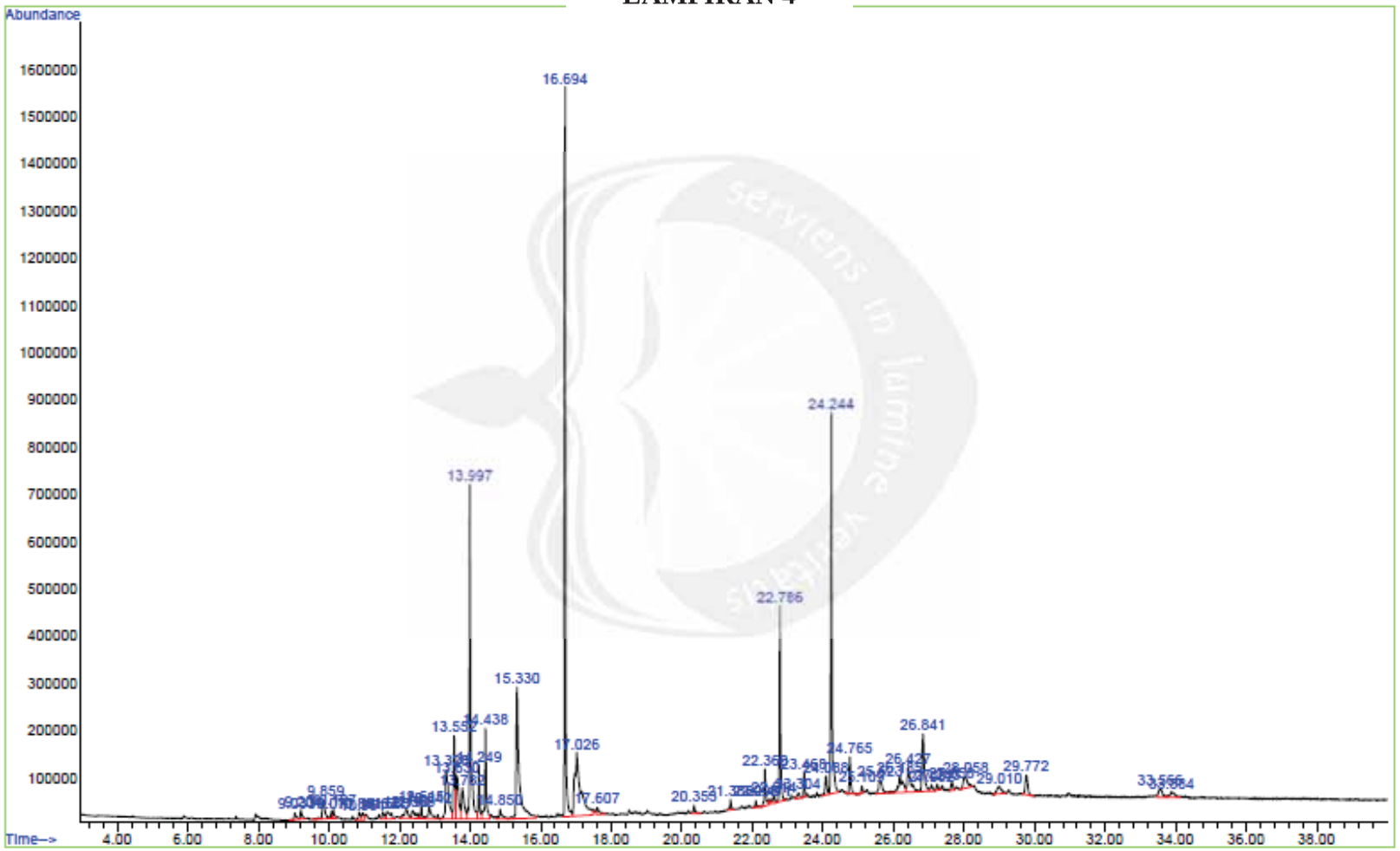
Duncan[a]

Perlakuan	N	Subset untuk alfa = 0,05	
		1	2
DMSO	5	,000000	
200 mg/ml	5	,000000	
100 mg/ml	5	,000000	
50 mg/ml	5	,000000	
25 mg/ml	5	,000000	
Ketoconazole 1% ($^{b}/_{v}$)	5		11,449000
Sig.		1,000	1,000

Rerata dari grup pada subset yang homogen ditampilkan.

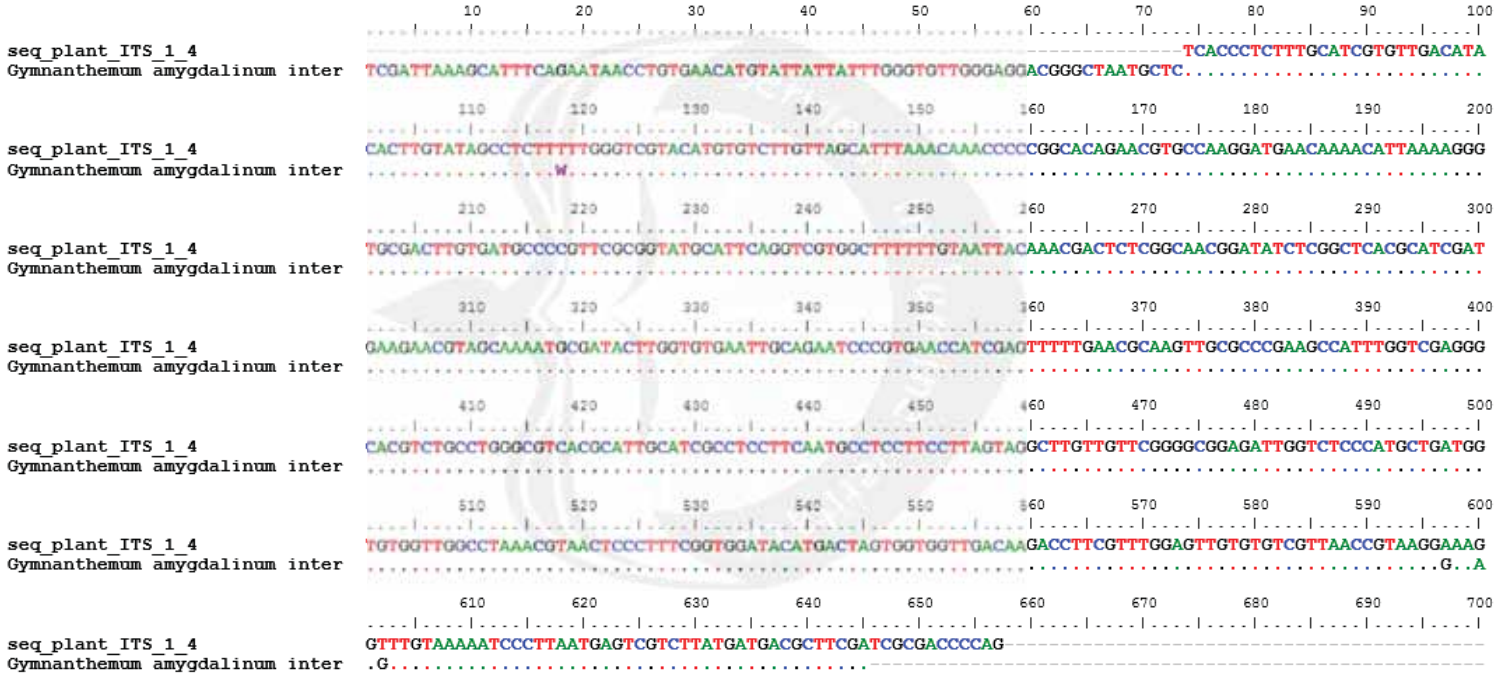
a. Penggunaan rerata ukuran sampel yang selaras = 5,000

LAMPIRAN 4



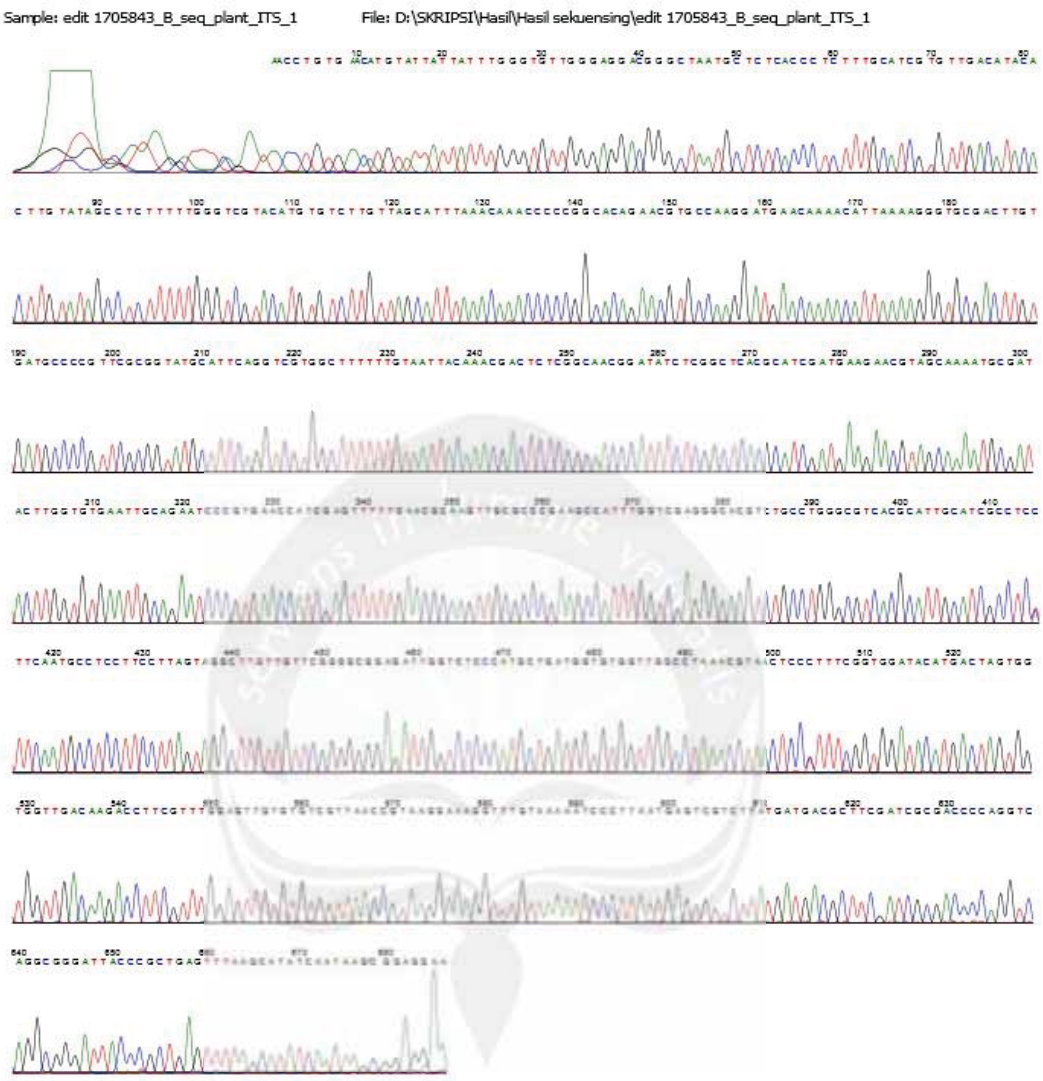
Gambar 17. Kromatogram Hasil Kromatogram Cair Ekstrak

LAMPIRAN 5



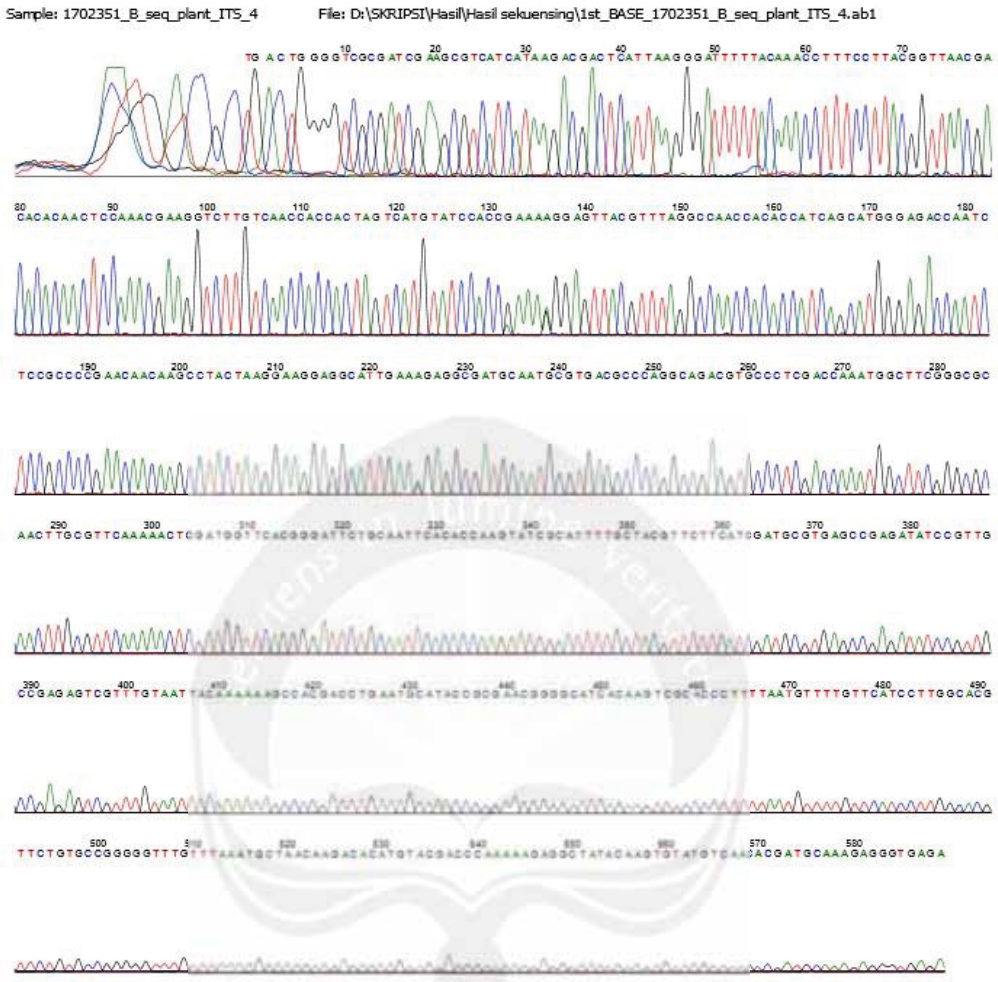
Gambar 18. Hasil *alignment* sekuens hasil *sekuensing* dengan sekuens *Gymananthemum amygdalinum* yang diperoleh dari NCBI Genbank

LAMPIRAN 6



Gambar 19. Kromatogram Sekuens DNA menurut Primer ITS 1

LAMPIRAN 7



Gambar 20. Kromatogram Sekuens DNA menurut Primer ITS 4

LAMPIRAN 8

BLAST®

Basic Local Alignment Search Tool

NCBI/ BLAST/ blastn suite/ **Formatting Results - 26S79TA0014**
Formatting options
Download
Blast report description

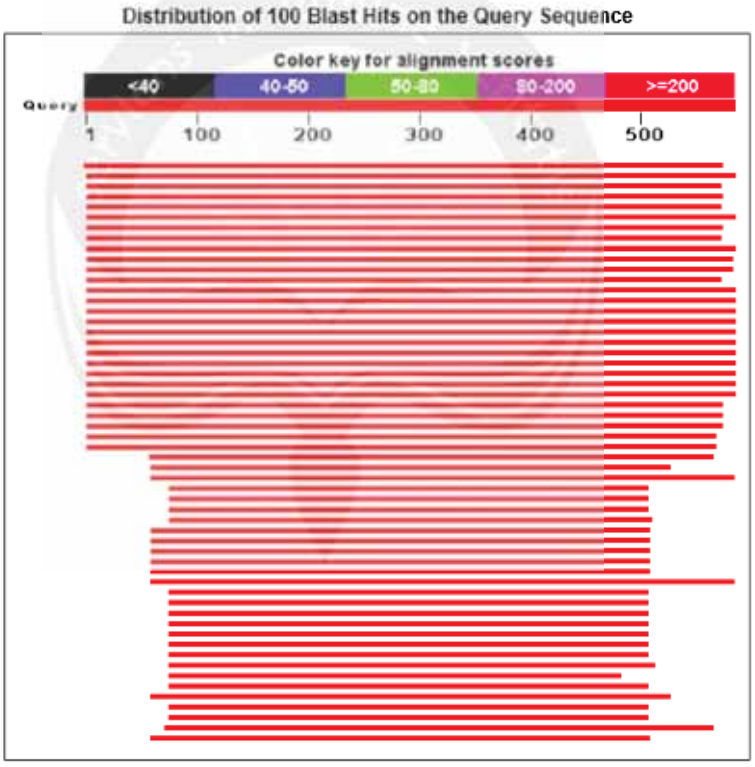
Nucleotide Sequence (585 letters)

RID 26S79TA0014 (Expires on 10-19 17:05 pm)

Query ID lcl|Query_133209
Description None
Molecule type nucleic acid
Query Length 585

Database Name nr
Description Nucleotide collection (nt)
Program BLASTN 2.2.32+

Graphic Summary



Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Gymnanthemum amygdalinum internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	1033	1033	97%	0.0	99%	AY504695.1
Gymnanthemum cylindriceps voucher S. Bunwong 378 (KKU) internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	898	898	99%	0.0	95%	HQ158404.1
Gymnanthemum corymbosum isolate K324 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	867	867	97%	0.0	94%	JN715898.1
Vernonia humblotii isolate Vhumblotii490 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	861	861	97%	0.0	94%	EF155819.1
Vernonia colorata isolate K383 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	859	859	97%	0.0	94%	JN715896.1
Decaneuropsis eberhardtii voucher S. Bunwong 384 (KKU) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	857	857	99%	0.0	93%	HQ158397.1
Gymnanthemum mespilifolium isolate GymanthemumK112 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	856	856	97%	0.0	94%	EF155775.1
Gymnanthemum mespilifolium isolate K112 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	854	854	97%	0.0	94%	JN715897.1
Vernonia cumingiana genomic DNA containing 18S rRNA gene, ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene, specimen voucher HITBC:Liana Mengsong 145_9_36	852	852	99%	0.0	93%	HG004807.1
Decaneuropsis cumingiana voucher S. Bunwong 74 (KKU) 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	841	841	99%	0.0	93%	HQ158396.1
Pleurocarpaea denticulata isolate ASL460 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	756	756	99%	0.0	90%	JX564746.1
Pleurocarpaea denticulata isolate K163 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	756	756	97%	0.0	91%	JN715917.1
Hesperomannia arborescens clone HPDL_3897 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	719	719	99%	0.0	89%	JX444455.1

Alignments

Gymnanthemum amygdalinum internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence

Sequence ID: **gb|AY504695.1|** Length: 645 Number of Matches: 1
Range 1: 73 to 645

Score	Expect	Identities	Gaps	Strand	Frame
1033 bits(559)	0.0()	569/574(99%)	2/574(0%)	Plus/Plus	

Features:

```
Query  1    CTCACCCTCTTTGCATCGTGTTGACATACACTTGTATAGCCTCTTTTTGGGTCGTACATG  60
            ||||||||||||||||||||||||||||||||||||||||||| |||||||||||||||
Sbjct  73   CTCACCCTCTTTGCATCGTGTTGACATACACTTGTATAGCCTCTTWTTGGGTCGTACATG  132

Query  61   TGTCTTGTTAGCATTTAAACAAACCCCCGGCACAGAACGTGCCAAGGATGAACAAAACAT  120
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  133  TGTCTTGTTAGCATTTAAACAAACCCCCGGCACAGAACGTGCCAAGGATGAACAAAACAT  192

Query  121  TAAAAGGGTGCGACTTGTGATGCCCCGTTCGCGGTATGCATTCAGGTCGTGGCTTTTTTG  180
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  193  TAAAAGGGTGCGACTTGTGATGCCCCGTTCGCGGTATGCATTCAGGTCGTGGCTTTTTTG  252

Query  181  TAATTACAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGC  240
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  253  TAATTACAAACGACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAACGTAGC  312

Query  241  AAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAA  300
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  313  AAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGAACCATCGAGTTTTTGAACGCAA  372

Query  301  GTTGCGCCCGAAGCCATTTGGTCGAGGGCACGTCTGCCTGGGCGTCACGCATTGCATCGC  360
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  373  GTTGCGCCCGAAGCCATTTGGTCGAGGGCACGTCTGCCTGGGCGTCACGCATTGCATCGC  432

Query  361  CTCTTTCAATGCCTCCTTCCTTAGTAGGCTTGTTGTTCGGGGCGGAGATTGGTCTCCCAT  420
            ||| |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  433  CTCCTTCAATGCCTCCTTCCTTAGTAGGCTTGTTGTTCGGGGCGGAGATTGGTCTCCCAT  492

Query  421  GCTGATGGTGTGGTTGGCCTAAACGTAACTCCTTTTCGGTGGATACATGACTAGTGGTGG  480
            ||||||||||||||||||||||||||| ||||||||||||||||||||||||||||||||
Sbjct  493  GCTGATGGTGTGGTTGGCCTAAACGTAACTCCCTTTCGGTGGATACATGACTAGTGGTGG  552

Query  481  TTGACAAGACCTTCGTTTGGAGTTGTGTGTCGTTAACCGTAAGG-AAAGGTTTGTAAAAA  539
            ||||||||||||||||||||||||||||||||||||||||||| ||||| |||||||||
Sbjct  553  TTGACAAGACCTTCGTTTGGAGTTGTGTGTCGTTAACCGTAAGGGAAAGG-TTGTAAAAA  611

Query  540  TCCCTTAATGAGTCGTCTTATGATGACGCTTCGA  573
            |||||||||||||||||||||||||||||||||
Sbjct  612  TCCCTTAATGAGTCGTCTTATGATGACGCTTCGA  645
```

Gambar 21. BLAST Sekuens DNA *Nan Fei Shu*

LAMPIRAN 9

Sekuens Gymnanthemum amygdalinum yang diperoleh dari NCBI GenBank (fasta format)

>gi|40748098|gb|AY504695.1| Gymnanthemum amygdalinum internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence

TCGATTAAAGCATTTCAGAATAACCTGTGAACATGTATTATTAT
TTGGGTGTTGGGAGGACGGGCTAATGCTCTCACCCTCTTTGCAT
CGTGTTGACATACACTTGTATAGCCTCTTWTTGGGTCGTACATG
TGTCTTGTTAGCATTTAAACAAACCCCCGGCACAGAACGTGCCA
AGGATGAACAAAACATTAAAAGGGTGCGACTTGTGATGCCCCG
TTCGCGGTATGCATTCAGGTCGTGGCTTTTTTGTAATTACAAAC
GACTCTCGGCAACGGATATCTCGGCTCACGCATCGATGAAGAA
CGTAGCAAAATGCGATACTTGGTGTGAATTGCAGAATCCCGTGA
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CAATGCCTCCTTCCTTAGTAGGCTTGTTGTTCGGGGCGGAGATT
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